

IGe ANTIBODIES TO *PLASMODIUM FALCIPARUM* AND SEVERITY OF MALARIA IN CHILDREN OF ONE ETHNIC GROUP LIVING IN BURKINA FASO

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Abstract. *Plasmodium falciparum* malaria infection induces elevated blood levels of both total immunoglobulin and anti-plasmodial antibodies belonging to different isotypes. We have previously shown that donors living in areas of malaria transmission develop malaria-specific IgE antibodies that are present at highest concentrations in patients with severe disease, suggesting a role for this isotype in malaria pathogenesis. To establish the possible importance of IgE in the course and severity of this disease, we have analyzed a large and homogenous group of African children (age range = 6 months to 15 years) belonging to one ethnic group (Mossi) living in identical epidemiologic conditions in the same urban area (Ouagadougou) of Burkina Faso. While IgG antibodies to *P. falciparum* increased to high concentrations in very young children and then remained at these levels in older patients, IgE antibodies increased with age, becoming most significantly elevated in children more than four years of age. In older children, those with severe malaria had significantly higher IgE antibody levels than those with non-severe disease. No significant differences between the patient groups were seen for IgG antibodies to *P. falciparum*. However, when the patients with severe malaria were divided into two groups distinguished by the presence of absence of coma, both IgG and IgE antibodies against malaria were lower in the comatous patients than in the non-comatous patients. The results support the conclusion that IgE antibodies against malaria, regardless of their possible protectivity, also contribute to disease severity in this large and homogenous group of African children.

INTRODUCTION

People living in malaria-endemic regions develop elevated levels of IgE.¹ This is probably due to exposure to several parasitic infections, but evidence from experimental and *in vivo* malaria indicate that *Plasmodium per se* can give rise to IgE.² In schistosomiasis, a link between the presence of specific IgE antibodies to *Schistosoma* and the acquisition of immunity against the infection has been demonstrated.³ In other helminthic infections, a protective role of IgE has been suggested.^{4,5} In general, elevated levels of IgE reflect an underlying imbalance in the ratio of T helper (Th) cells in favor of Th2 cells producing interleukin-4 (IL-4) and IL-13, which are responsible for IgM/IgG switching to IgE.^{6–8} Production of IL-4 by T cells from donors primed by natural malaria infection was found to be associated with elevated antibody levels specific for *P. falciparum* antigens.⁹ It was shown that the levels of IgE malarial antibodies in *P. falciparum*-primed individuals reflected an increased ratio of IL-4- to interferon-gamma (IFN- γ)-producing cells.¹⁰ Further studies of IgE in immunity to malaria suggested that total and specific IgE antibodies to *P. falciparum* could have a pathogenic role during malaria infection.^{11,12} One of the possible pathogenic mechanism may involve the deposition of IgE in basement membranes of cerebral capillaries¹³ when *P. falciparum*-infected erythrocytes adhere to endothelial cells lining capillaries and postcapillary venules.^{14,15} Such IgE deposits may contain malaria antigen and/or consist of IgE complexed with IgG anti-IgE antibodies that also occur at elevated levels in patients with *P. falciparum* malaria. These deposits may induce local overproduction of tumor necrosis factor- α (TNF- α),⁸ thereby contributing to the development and outcome of cerebral malaria.^{16,17}

In this study, we analyzed the role of IgE in the clinical outcome of malaria infection in patients living in the same

epidemiologic context and belonging to the same ethnic group. Total and specific IgE antibodies to *P. falciparum* were compared with age-matched malaria patients with different clinical presentations of the disease. In parallel, IgG antibodies to *P. falciparum* were also evaluated.

MATERIALS AND METHODS

Study area and patients. Patients analyzed in the present work were randomly selected from a large epidemiologic study of severe malaria performed in Burkina Faso.¹⁸ This study was conducted during two high transmission seasons for malaria (1993 and 1994) at the 158-bed pediatric ward of the Ouagadougou University Hospital. To limit the possible influence of confounding factors such as genetic background and history of exposure to malaria, only subjects belonging to the Mossi ethnic group and coming from the urban area of Ouagadougou were included in this analysis. Moreover, as an additional check for possible differences in anti-malaria immunity related to variations in malaria exposure, the humoral immune response against the repetitive domain of the *P. falciparum* circumsporozoite protein (CSP)¹⁸ was determined in all patients.

The study area is characterized by a rainy season from June to October, which corresponds to the high transmission season for malaria, and by a long dry season from November to May. The urban area is characterized by entomologic inoculation rates from one to ten per person per year^{19,20} and the main malaria vectors are *Anopheles gambiae*, *A. arabiensis* and *A. funestus*.²¹

The protocol of the study was reviewed and approved by the Centre National de Lutte contre le Paludisme of the Ministry of Health of Burkina Faso. On admission and after oral informed consent of the parents, children were weighed and a

venous blood sample was drawn for measurement of parasitemia, blood glucose levels, plasma creatinine and hemoglobin concentrations, hematocrit, and complete blood cell count. Plasma was separated and transferred into sterile tubes containing tripotassium EDTA and kept at -20°C until serologic tests were done.

Children (age range = 6 months to 15 years) were included in the study. As previously reported,¹⁸ severe malaria was defined by the presence of *P. falciparum* in the a thick blood film associated with at least one of the following conditions: prostration (incapacity of the child to sit without help, in the absence of coma), unrousable coma (score between 0 and 2 on the Glasgow modified coma scale), repeated generalized convulsions (more than two episodes in the preceding 24 hours), severe anemia (hemoglobin level < 5 g/dL), hypoglycemia (glucose level < 40 mg/dL), pulmonary edema/respiratory distress, spontaneous bleeding, and renal failure (plasma creatinine > 3 mg/dL). Children with other detectable infections or other causes of clinical presentation were not included in the study. Non-complicated malaria was defined as a clinical illness characterized by an axillary temperature > 37.5°C associated with a *P. falciparum*-positive blood film. Patients with patent bacterial infections were not included in the study.

Patients were treated according to World Health Organization guidelines with a complete regimen of drugs that were provided free of charge as a part of the study.

Blood examination. Thick and thin blood smears were prepared following standard procedures and 100 microscopic fields of the thick blood smears were examined (approximately 20 leukocytes/field at a magnification of 1,000 = approximately 0.25 µL of blood). The *Plasmodium* species was identified on thin blood smears.

Parasite extract preparation. Lysates of mature stages of percoll-enriched *P. falciparum*-infected erythrocytes of the laboratory strain F32 were prepared and used as antigen for the detection of malarial antibodies as described elsewhere.²²

Serologic tests. Levels of total IgE and anti-*P. falciparum* IgE or IgG were determined by an enzyme-linked immunosorbent assay (ELISA) as previously described.¹¹ Briefly, high-binding, flat-bottomed, 96-well micro-ELISA plates (Costar; Corning Inc. Life Sciences, Acton, MA) were coated overnight with 50 µL/well of affinity-purified IgG fractions of rabbit anti-human IgE (Miab, Uppsala, Sweden) at a concentration of 5 µg/mL for total IgE and with 50 µL/well of the parasite antigen (10 µg/mL). After three hours of incubation, plates were blocked with 0.5% bovine serum albumin in phosphate-buffered saline at 37°C to avoid non-specific binding. Samples were then added at different dilutions: 1:1,000 for the determination of total IgE and anti-*P. falciparum* IgG and 1:100 for anti-*P. falciparum* IgE. Samples were tested in duplicate. The sera were allowed to react for one hour at room temperature for all determinations, with the exception of anti-*P. falciparum* IgE, which were incubated overnight. Bound IgG was then detected by adding goat-anti human IgG conjugated to alkaline phosphatase (ALP) (Mabtech, Nacka, Sweden). Total IgE and bound anti-*P. falciparum* IgE were detected with biotinylated rabbit anti-human IgE diluted 1:1,000, followed by ALP-conjugated streptavidin (diluted 1:2,000) (Mabtech, Stockholm, Sweden) as described in detail elsewhere.¹¹ The optical density values were read in a Vmax Microplate Reader (Molecular Devices Corporation, Sunny-

vale, CA) at 405 nm wavelength. The concentrations of IgG and IgE antibodies and of total IgE were calculated from standard curves obtained by incubating the coated plates with five serial dilutions of either affinity-purified human serum IgE (National Institute for Biological Standards and Control, Hertfordshire, United Kingdom) or highly purified IgG (Jackson Immuno Research Laboratories, West Grove, PA).⁸ The Ig levels are given as geometric means of the concentrations found in all donors with either non-complicated or severe malaria (with and/or without coma) as indicated.

The CSP antibody response was evaluated with a Sclavo (Sovicille, Italy) ELISA Kit based on (NANP)₄₀ antigen.²³ The cut-off value for seropositivity for antibody to CSP was the geometric mean + three standard deviations of the values obtained with sera from 30 non-malaria-exposed Italian donors. Levels of antibody to CSP were expressed as the log₁₀ of arbitrary immuno-enzymatic units, using a reference curve obtained by serial dilutions of a pool of human sera highly reactive to *P. falciparum* CSP.

RESULTS

The characteristics of the patients included in this study are shown in Table 1. A total of 661 patients were analyzed, 317 with a clinical picture of severe malaria including 163 comatous patients and 344 with non-complicated malaria. The mean age was comparable among the different clinical groups. Analysis of the humoral immune response against the repetitive domain of *P. falciparum* CSP, a good indicator of malaria exposure,¹⁹ revealed no significantly different antibody levels between the clinical groups; similarly, no differences were recorded in terms of anti-CSP IgG prevalences. This data suggest that the patients had been exposed to similar malaria transmission levels. No significant differences were recorded when comparing *P. falciparum* parasite densities (Table 1). The lack of a clear cut association between *P. falciparum* densities and severity of disease is consistent with previous observations.²⁴

Six hundred sixty-one of the patients were analyzed for total IgE and 647 for anti-*P. falciparum* IgE. Four hundred sixty-six of these patients were also analyzed for anti-*P. falciparum* IgG. Consistent with previous studies,¹² no significant differences were observed when comparing total IgE levels between patients with non-severe or severe malaria, respectively. This was true for severe disease patients both with and without coma. Among the severely ill patients, both anti-*P. falciparum* IgE and IgG levels were lowest in the co-

TABLE 1

Characteristics of malaria patients tested for parasite densities and levels of IgG anti-*Plasmodium falciparum* CSP antibodies at the Ouagadougou University Hospital, Burkina Faso, during the high malaria transmission season in 1993 and in 1994*

Malaria patients	No.	Age, years (mean ± SE)	Anti-CSP IgG (IEU/ml)	Parasites/µL (geometric mean)
Nonsevere	344	5.61 ± 0.18	0.036	15,274
Severe, non-comatous	154	5.57 ± 0.28	0.032	17,349
Severe, comatous	163	5.03 ± 0.21	0.032	12,221

* The patients were divided into two age groups (0.7–4.0 and 4.1–15 years, respectively). CSP = circumsporozoite protein; IEU = immunoenzymatic units (see Reference 23).

matous patients group (0.565 ng/mL and 8.746 µg/mL, respectively), but highest in the severe malaria patients without coma (0.720 ng/mL or 13.17 µg/mL, respectively). These differences (comatous versus non-comatous patients) within the severe disease group were statistically significant (anti-*P. falciparum* IgE; $P \leq 0.02$ and anti-*P. falciparum* IgG; $P \leq 0.01$).

To investigate the effects of age when comparing antibody levels and disease severity, we divided a randomly chosen group of patients into two age groups (Table 2). Anti-*P. falciparum* IgG levels were high already in the youngest age group (≤ 8 years old; Figure 1a). The apparent increase in concentrations above this age was not statistically significant. Similar findings were also made for antibodies to CSP that reflect exposure to malaria. Similar to what had been found in Thailand¹² for anti-*P. falciparum* IgG, there were no significant differences between severe and non-severe cases regardless of age. However, the 4–15-year-old patients with severe malaria and coma appeared to have lower levels of anti-malarial IgG than those without coma (Table 2).

In the 0.5–4.0-year-old children, IgE antibody levels were very low (Figure 1b), but appeared to be somewhat higher in the patients with non-complicated malaria than in those with severe malaria ($P \leq 0.05$; Table 2). However, the possible biologic significance of this finding is uncertain. On the other hand the 4–15-year-old patients with severe malaria had significantly higher levels of anti-*P. falciparum* IgE than the 4–15-year-old patients with non-severe malaria. This was true for both comatous and non-comatous patients. For total IgE levels, the differences in concentrations between the non-severe or severe groups and comatous patients were statistically significant in children more than four years old (Table 2).

DISCUSSION

In malaria-endemic regions, the levels of antibodies against malaria increase with age as a reflection of exposure to parasite antigens. The increase in antibody levels is usually accompanied by a decreased risk to develop severe clinical symptoms of malaria. The role of anti-*P. falciparum* IgG in protection against malaria had been documented nearly 40

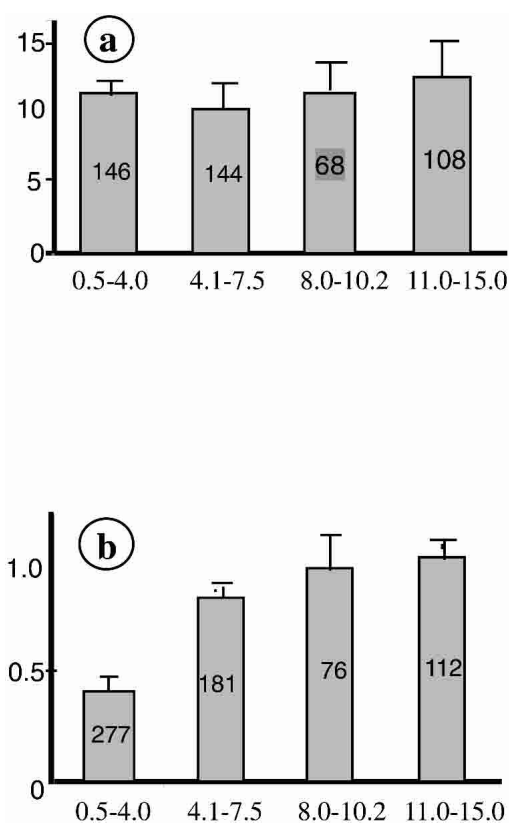


FIGURE 1. Geometric mean concentrations of antibodies to *Plasmodium falciparum* in different age groups in Burkina Faso. The numbers inside the columns show the number of patients. Age ranges (in years) are shown along the x-axes. Error bars show the mean + SEM. **a**, IgG antibodies (µg/mL). **b**, IgE antibodies (ng/mL).

TABLE 2

Total IgE, anti-*Plasmodium falciparum* IgE, and anti-*P. falciparum* IgG antibodies in patients with different clinical presentations of malaria*

		Total IgE (ng/ml)		IgE antibodies (ng/ml)		IgG antibodies (µg/ml)	
		Age group, years		Age group, years		Age group, years	
Malaria patients		0.5-4.0	4.1-15	0.5-4.0	4.1-15	0.5-4.0	4.1-15
Nonsevere	GM	228	174	0.47	0.78	11.13	11.25
	No.	138	207	133	203	70	157
Severe, all	GM	227	257	0.25	1.06	10.13	10.50
	P	NS	<0.05	<0.05	<0.001	NS	NS
Severe, non-comatous	No.	150	167	144	167	76	163
	GM	235	223	0.40	1.11	14.81	12.76
Severe, non-comatous	P	NS	NS	NS	<0.001	NS	NS
	No.	68	86	64	86	23	86
Severe, comatous	GM	221	229	0.32	1.01	9.20	8.45
	P	NS	<0.01	<0.01	<0.01	NS	NS
Severe, comatous	No.	82	81	80	81	53	77

* The patients were divided into two age groups (0.5-4.0 and 4.1-15 years, respectively). P values, by Student's *t*-test, are for differences between non-severe and severe (all, non-comatous, or comatous) patients. GM = geometric mean concentration; No. = number of patients; NS = not significant.

years by passive transfer experiments and in epidemiologic studies.^{25,26} Anti-malarial IgE may well be protective, but may also contribute to severity of the disease. Thus, anti-plasmodial IgG and IgE that react with the same plasmodial antigens¹¹ exert contrasting functions during malaria infection, and the IgG:IgE antibody ratio appears to be an esti-

mate of this balance between protectivity and pathogenicity.¹² Consistent with this finding, the comatous patients in this study, who tended to have the lowest levels of anti-malarial IgG when compared with severe disease patients without coma, also had the lowest IgG:IgE ratios. These results suggest that comatous patients in general may have a weaker IgG response than those with severe disease without coma.

Although anti-malarial IgG appears to play a role in protection against malarial disease,^{25,26} there is frequently no clear correlation between total antibody levels and the severity of clinical symptoms.^{24,27} As pointed out earlier in this report, the lowest levels of anti-*P. falciparum* IgG were found in comatous patients. If confirmed by further studies, low IgG antibody levels in comatous patients could be due to a weak humoral response. Alternatively, in patients with coma, a higher amount of antibody may be bound to sequestered parasites. An additional (but not exclusive) explanation could be that these differences in antibody concentrations reflect other host genetic factors contributing to protection and/or pathogenesis of disease. This suggestion would be compatible with recently published results that higher levels of anti-*P. falciparum* IgG in a more protected sympatric ethnic group, the Fulanis in west Africa, were associated with the IL-4-524 T allele.²⁸

The increase in levels of IgE antibodies with age supports the conclusion that they may play a role in protection against malaria, as previously suggested by others.^{29,30} This has also been well established for schistosomiasis.^{3-5,31} The higher IgE antibody concentrations in non-comatous patients compared with comatous patients may also support a protective role of these antibodies. However, as previously documented, IgE also has a part in the pathogenesis of severe malaria.¹⁰⁻¹² In the present study, we show that for patients more than four years of age the *Plasmodium*-specific IgE antibody concentrations may have become sufficiently high to contribute to a more severe clinical picture (Table 2). It is tempting to speculate that genetic polymorphism in the IL-4 promotor²⁸ (Gyan B and others, unpublished data) might lead to elevated levels of IgE antibodies, which in turn will promote the production of TNF and/or nitric oxide. Individuals carrying genes linked to over-production of these factors may then be afflicted by more severe disease. Further studies using genetically defined patients are needed to establish this possibility.

Received June 5, 2002. Accepted for publication April 12, 2003.

Acknowledgements: We thank all the children and parents who participated in this study for their understanding and assistance. We are indebted to the pediatric and laboratory staff of the Centre Hospitalier National Yalgado Ouedraogo in Ouagadougou, Burkina Faso, for continuous and skilful technical assistance. This study was conducted at the Centre National de Recherche et Formation sur le Paludisme of the Ministry of Health of Burkina Faso. The excellent technical assistance of M. Hagstedt is gratefully acknowledged.

Financial support: This study was supported by the Programma di Assistenza Tecnica della Direzione Generale e per la Cooperazione allo Sviluppo of the Italian Ministry of Foreign Affairs, the Swedish Development Agency (Sida/SAREC), the Swedish Medical Research Council (MFR), the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR), the Bergvalls Foundation, and the International Collaboration with Developing Countries (INCO-DC) European Union contract no. IC 18-CT 980361.

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